

What is claimed is:

1. A method of non-enzymatic ligation of a nucleic acid, comprising contacting a polynucleotide-3' phosphorothiolate with an acceptor polynucleotide under
5 conditions that allow formation of a phosphodiester bond between said polynucleotide-3' phosphorothiolate and said acceptor polynucleotide.

2. The method of claim 1, wherein said polynucleotide-3' phosphorothiolate further comprises a 3'
10 SNP moiety.

3. The method of claim 1, wherein said polynucleotide-3' phosphorothiolate further comprises a duplex polynucleotide.

4. The method of claim 1, wherein said acceptor
15 polynucleotide further comprises a duplex polynucleotide.

5. The method of claim 1, further comprising transducing into a host cell a polynucleotide-3' phosphorothiolate having a phosphodiester bond with said acceptor polynucleotide.

6. The method of claim 1, further comprising the step:

contacting a polynucleotide-3' phosphorothiolate precursor and an activator under
5 conditions sufficient to react said polynucleotide-3' phosphorothiolate precursor and said activator to produce said polynucleotide-3' phosphorothiolate.

7. The method of claim 6, wherein said activator
10 is iodonitrobenzene.

8. A method of molecular cloning comprising, contacting an insert comprising a polynucleotide-3' phosphorothiolate with an acceptor vector under conditions that allow formation of a phosphodiester bond between said
15 insert and said acceptor vector to generate a vector comprising an insert polynucleotide.

9. The method of claim 8, further comprising transforming said vector comprising an insert polynucleotide into a host cell.
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10. The method of claim 8, wherein said polynucleotide-3' phosphorothiolate further comprises a 3' SNP moiety.

11. The method of claim 8, further comprising the
25 step:

contacting a polynucleotide-3' phosphorothiolate precursor and iodonitrobenzene under conditions sufficient to react said polynucleotide-3'

phosphorothiolate precursor and said iodonitrobenzene to produce said polynucleotide-3' phosphorothiolate.

12. A method of molecular cloning comprising,
5 contacting a vector comprising a polynucleotide-3' phosphorothiolate with an acceptor polynucleotide, under conditions that allow formation of a phosphodiester bond between said vector and said acceptor polynucleotide to generate a vector comprising said acceptor polynucleotide.

10 13. The method of claim 12, further comprising transforming said vector comprising said acceptor polynucleotide into a host cell.

14. The method of claim 12, wherein said
15 polynucleotide-3' phosphorothiolate further comprises a 3' SNP moiety.

15. The method of claim 12, wherein said vector further comprises a 3' phosphorothiolate moiety at one or more terminal ends.

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16. The method of claim 12, further comprising the step:

contacting a polynucleotide-3' phosphorothiolate precursor and an activator under
25 conditions sufficient to react said polynucleotide-3' phosphorothiolate precursor and said activator to produce said polynucleotide-3' phosphorothiolate.

17. A kit, comprising:

- (a) a polynucleotide-3' phosphorothiolate; and
- (b) a buffer in an aqueous solution.

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18. The kit of claim 17, wherein said polynucleotide-3' phosphorothiolate further comprises one or more 3' phosphorothiolate moieties.

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19. The kit of claim 17, wherein said polynucleotide-3' phosphorothiolate further comprises a single stranded polynucleotide.

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20. The kit of claim 18, wherein said single stranded polynucleotide further comprises an oligonucleotide.

21. The kit of claim 17, wherein said polynucleotide-3' phosphorothiolate further comprises a duplex polynucleotide.

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22. The kit of claim 17, wherein said polynucleotide-3' phosphorothiolate comprises a 3'-SNP moiety.

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23. A kit, comprising:

- (a) a polynucleotide-3' phosphorothiolate precursor; and
- (b) an activator.

24. The kit of claim 23, wherein said polynucleotide-3' phosphorothiolate further comprises one or more 3' phosphorothiolate moieties.

5 25. The kit of claim 24, wherein said polynucleotide-3' phosphorothiolate further comprises a single stranded polynucleotide.

26. The kit of claim 24, wherein said single stranded polynucleotide further comprises an
10 oligonucleotide.

27. The kit of claim 24, wherein said polynucleotide-3' phosphorothiolate further comprises a duplex polynucleotide.

15 28. The kit of claim 24, wherein said polynucleotide-3' phosphorothiolate comprises a 3'-SNP moiety.

29. A method of ligating a nucleic acid, comprising contacting a polynucleotide-5' phosphorothiolate
20 with a non-sequence specific topoisomerase, or a fragment or modification thereof, and an acceptor polynucleotide under conditions that allow formation of a phosphodiester bond between said polynucleotide-5' phosphorothiolate and said acceptor polynucleotide, with the proviso that said
25 polynucleotide-5' phosphorothiolate does not contain the nucleotide sequence G(C/T)CCTT (SEQ ID NO:5).

30. The method of claim 29, wherein said topoisomerase is human topoisomerase I, or a fragment or modification thereof.

31. The method of claim 30, wherein said human
5 topoisomerase I is Topo65, or a fragment or modification thereof.

32. The method of claim 29, wherein said polynucleotide-5' phosphorothiolate further comprises a duplex polynucleotide.

10 33. The method of claim 29, wherein said acceptor polynucleotide further comprises a vector.

34. The method of claim 29, wherein said polynucleotide-5' phosphorothiolate further comprises a vector.

15 35. The method of claim 29, wherein said polynucleotide-5' phosphorothiolate further comprises a polynucleotide having a 5' phosphorothiolate moiety incorporated within four base pairs from a 3' end of said polynucleotide-5' phosphorothiolate.

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36. A kit, comprising:

- (a) a polynucleotide-5' phosphorothiolate, with the proviso that said polynucleotide-5' phosphorothiolate does not contain a nucleotide sequence selected from the group of SEQ ID NO:5, SEQ ID NO:6 or SEQ ID NO:7;
- (b) a non-sequence specific topoisomerase, or fragment or modification thereof having topoisomerase activity.

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37. The kit of claim 36, wherein said topoisomerase is human topoisomerase I, or a fragment or modification thereof.

38. The kit of claim 37, wherein said topoisomerase is Topo65, or a fragment or modification thereof.

39. The kit of claim 36, wherein said polynucleotide-5' phosphorothiolate is a single stranded polynucleotide-5' phosphorothiolate.

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40. The kit of claim 37, wherein said single stranded polynucleotide-5' phosphorothiolate further comprises an oligonucleotide.

41. The kit of claim 36, wherein said polynucleotide-5' phosphorothiolate further comprises a duplex polynucleotide-5' phosphorothiolate.

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42. The kit of claim 41, wherein said duplex polynucleotide-5' phosphorothiolate further comprises one or more terminal end overhangs.

43. The kit of claim 42, wherein said one or more
5 terminal end overhangs further comprise a nucleotide sequence complementary to one or more restriction endonuclease cleavage sites.

44. A composition comprising,
10 (a) a polynucleotide-5' phosphorothiolate, with the proviso that said polynucleotide-5' phosphorothiolate does not contain a nucleotide sequence selected from the group of SEQ ID NO:5, SEQ ID NO:6 or SEQ ID NO:7; and
15 (b) a non-sequence specific topoisomerase, or fragment or modification thereof having topoisomerase activity.

45. The composition of claim 44, wherein said
20 topoisomerase is human topoisomerase I, or a fragment or modification thereof.

46. The composition of claim 45, wherein said topoisomerase is Topo65, or a fragment or modification thereof.

25 47. The composition of claim 44, wherein said polynucleotide-5' phosphorothiolate is a single stranded polynucleotide-5' phosphorothiolate.

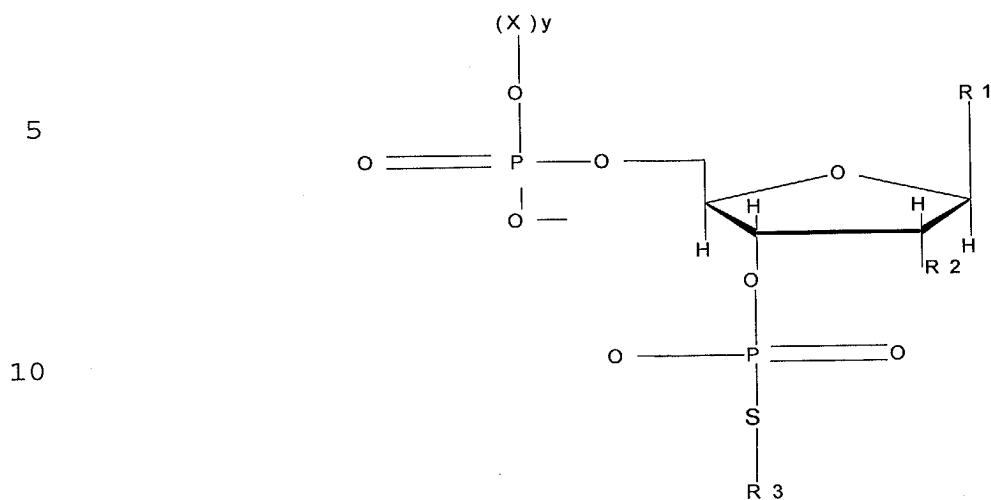
48. The composition of claim 47, wherein said single stranded polynucleotide-5' phosphorothiolate further comprises an oligonucleotide.

5 49. The composition of claim 44, wherein said polynucleotide-5' phosphorothiolate further comprises a duplex polynucleotide-5' phosphorothiolate.

10 50. The composition of claim 49, wherein said duplex polynucleotide-5' phosphorothiolate further comprises one or more terminal end overhangs.

51. The composition of claim 50, wherein said one or more terminal end overhangs further comprise a nucleotide sequence complementary to one or more restriction endonuclease cleavage sites.

52. A compound of the formula:



wherein,

X is a nucleotide;

y is a positive integer;

R1 is a nucleotide base;

R2 is H or OH; and

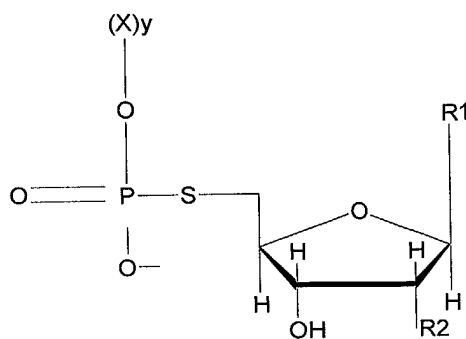
R3 is a halo, alkyl, substituted alkyl,
sulfonate moiety, phenyl,
substituted phenyl.

53. The compound of claim 52, wherein
R2 is H.

54. The compound of claim 52, wherein
R3 is nitrophenyl.

25 55. The compound of claim 52, further comprising
a complementary polynucleotide.

56. A compound of the formula:



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wherein,

X is a nucleotide;

y is a positive integer;

R1 is cytosine or guanine; and

R2 is H or OH.

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